

Reduced MTHFD1 Activity in Male Mice Perturbs Folate- and Choline-Dependent One-Carbon Metabolism as Well as Transsulfuration^{1,2}

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Abstract

Impaired utilization of folate is caused by insufficient dietary intake and/or genetic variation and has been shown to prompt changes in related pathways, including choline and methionine metabolism. These pathways have been shown to be sensitive to variation within the *Mthfd1* gene, which codes for a folate-metabolizing enzyme responsible for generating 1-carbon (1-C)-substituted folate derivatives. The *Mthfd1*^{gt/+} mouse serves as a potential model of human *Mthfd1* loss-of-function genetic variants that impair MTHFD1 function. This study investigated the effects of the *Mthfd1*^{gt/+} genotype and folate intake on markers of choline, folate, methionine, and transsulfuration metabolism. Male *Mthfd1*^{gt/+} and *Mthfd1*^{+/+} mice were randomly assigned at weaning (3 wk of age) to either a control (2 mg/kg folic acid) or folate-deficient (0 mg/kg folic acid) diet for 5 wk. Mice were killed at 8 wk of age following 12 h of food deprivation; blood and liver samples were analyzed for choline, methionine, and transsulfuration biomarkers. Independent of folate intake, mice with the *Mthfd1*^{gt/+} genotype had higher hepatic concentrations of choline ($P = 0.005$), betaine ($P = 0.013$), and dimethylglycine ($P = 0.004$) and lower hepatic concentrations of glycerophosphocholine ($P = 0.002$) relative to *Mthfd1*^{+/+} mice. *Mthfd1*^{gt/+} mice also had higher plasma concentrations of homocysteine ($P = 0.0016$) and cysteine ($P < 0.001$) as well as lower plasma concentrations of methionine ($P = 0.0003$) and cystathionine ($P = 0.011$). The metabolic alterations observed in *Mthfd1*^{gt/+} mice indicate perturbed choline and folate-dependent 1-C metabolism and support the future use of *Mthfd1*^{gt/+} mice as a tool to investigate the impact of impaired 1-C metabolism on disease outcomes. J. Nutr. 143: 41–45, 2013.

Introduction

The *Mthfd1* gene encodes a trifunctional folate-metabolizing enzyme, C1-tetrahydrofolate (THF)⁵ synthase, which plays an important role in both nucleotide synthesis and the methionine cycle. The C1THF synthase enzyme [commonly referred to as methylenetetrahydrofolate dehydrogenase 1 (MTHFD1)] contains a synthetase activity that catalyzes the ATP-dependent conversion of formate and THF to 10-formylTHF, a cyclohydrolase activity that catalyzes the interconversion of 10-formylTHF and 5,10-methenylTHF, and a dehydrogenase activity that reduces 5,10-methenylTHF to 5,10-methyleneTHF (1) (Fig. 1).

A product of the C1THF synthase-catalyzed reactions, 5,10-methyleneTHF, exists at a branch point in the folate metabolic

pathway. 5,10-MethyleneTHF is a 1-carbon (1-C) donor for the de novo synthesis of thymidylate or alternatively can be irreversibly reduced to 5-methylTHF by the enzyme 5,10-methylenetetrahydrofolate reductase (1). 5-MethylTHF is a key methyl donor for homocysteine remethylation to methionine, a reaction that is functionally redundant with the betaine:homocysteine methyltransferase-catalyzed conversion of homocysteine to methionine (2–4). Both folate-mediated 1-C metabolism and choline degradation can independently supply 1-C units for homocysteine remethylation and therefore these 2 pathways are highly interrelated. Consequently, changes in either folate or choline status can result in commensurate changes in the status of the other nutrient, as shown in several rodent models (5–8) and human studies (9–11).

We recently generated and characterized a mouse with a gene-trap insertion in the 10-formylTHF synthetase domain of the *Mthfd1* gene (12). The *Mthfd1*^{gt/gt} genotype is embryonic lethal, but *Mthfd1*^{gt/+} mice are viable and fertile. The C1THF synthase enzyme produced from the gene-trap allele lacks synthetase activity and tissues from *Mthfd1*^{gt/+} mice have 50% lower total C1THF synthase protein. *Mthfd1*^{gt/+} mice exhibited perturbed 1-C metabolism and these aberrations were exacerbated by a diet

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⁵ Abbreviations used: AdoMet, S-adenosylmethionine; 1-C, 1-carbon; CBS, cystathionine β -synthase; FD, folate deficient; MTHFD, methylenetetrahydrofolate dehydrogenase; SNP, single nucleotide polymorphism; THF, tetrahydrofolate.

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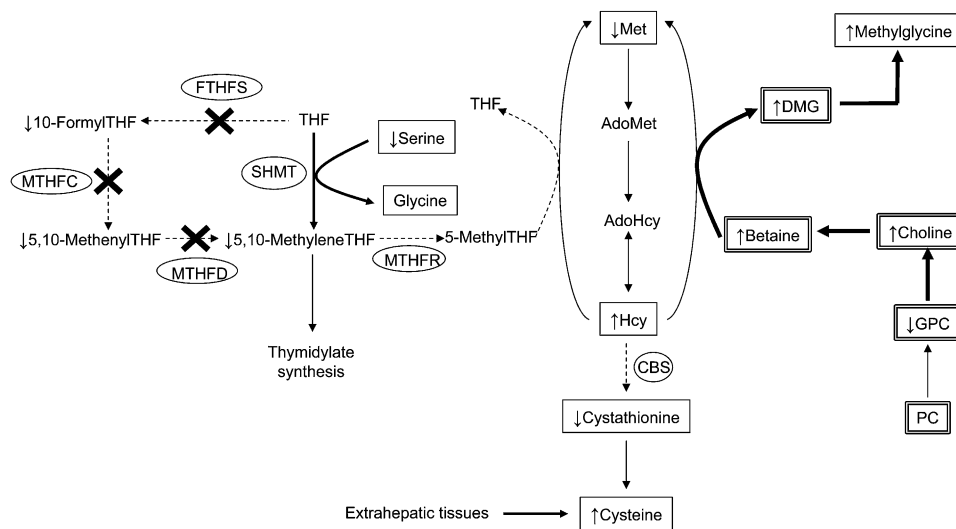


FIGURE 1 A working model of the metabolic effects of *Mthfd1* deficiency on choline- and folate-mediated 1-C metabolism. The product of the *Mthfd1* gene is C1THF synthase, which contains FTHFS, MTHFC, and MTHFD enzymatic activities. The “X” indicates enzymatic activities that are reduced by 50% in the *Mthfd1^{gt/+}* mouse model. Boxed metabolites are those that were measured in this study: a double-lined box indicates that the metabolite was measured in the liver, a single-lined box indicates that the metabolite was measured in plasma. Thicker arrow, process enhanced by reduced MTHFD1 activity; dashed arrow, process attenuated by reduced MTHFD1 activity. AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; 1-C, 1-carbon; CBS, cystathionine β -synthase; DMG, dimethylglycine; FTHFS, 10-formyltetrahydrofolate synthetase; GPC, glycerophosphocholine; Hcy, homocysteine; Met, methionine; MTHFC, methenyltetrahydrofolate cyclohydrolase; MTHFD, methylenetetrahydrofolate dehydrogenase; MTHFR, 5,10-methylenetetrahydrofolate reductase; PC, phosphatidylcholine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate.

deficient in both folate and choline (12). As such, the *Mthfd1^{gt/+}* mouse may serve as a model to investigate physiological outcomes of interactions between *MTHFD1* deficiency in humans and nutrients with key roles in 1-C metabolism. The *MTHFD1* G1958A single nucleotide polymorphism (SNP) (rs2236225, R653Q) results in a thermolabile protein with reduced synthetase activity (13) and is associated with increased risk for neural tube defects, fetal loss, and breast and gastric cancers (14–18). Carriers of the 1958A allele are also shown to exhibit increased circulating levels of homocysteine and impaired methionine cycle function (18,19) as well as increased risk of choline deficiency and organ dysfunction (20). Similarly, a recently identified inborn error of metabolism in which the patient inherited 2 deleterious SNPs in *MTHFD1* results in megaloblastic anemia, hyperhomocysteinemia, and severe combined immunodeficiency (21).

The primary aim of the current study was to quantify the effects of the *Mthfd1^{gt/+}* genotype on biomarkers of choline metabolism. Because our previous study used a diet that was deficient in both folate and choline, the current study sought to explore the implications of *Mthfd1* disruption on 1-C metabolism under conditions of dietary folate deficiency alone.

Materials and Methods

Experimental mice and diets. All study protocols were approved by the Institutional Animal Care and Use Committee of Cornell University and conform to the NIH Guide for the Care and Use of Laboratory Animals. Study mice were generated by crossing C57Bl/6 female mice to 129P2/OlaHsd *Mthfd1^{gt/+}* male mice. C57Bl/6 *Mthfd1^{gt/+}* mice were previously described (12). At weaning, male offspring were randomly assigned to either an AIN-93G diet (22) (control diet, Dyets) that contained 2 mg/kg folic acid or to a modified AIN-93G diet lacking folic acid [folate-deficient (FD) diet, Dyets]. All mice were fed the respective diets for 5 wk postweaning. Experimental mice were genotyped as described elsewhere (12).

Tissue harvest. Mice were killed by cervical dislocation after 12 h of food deprivation. Blood was collected via cardiac puncture into heparin-

coated tubes. Plasma was separated by centrifugation and snap frozen in liquid nitrogen. Liver samples were rinsed with PBS and snap frozen in liquid nitrogen, then stored at -80°C prior to choline analysis.

Analysis of plasma metabolites. Plasma total homocysteine, cystathionine, total cysteine, methionine, serine, glycine, α -aminobutyric acid, N, N-dimethylglycine, and N-methylglycine were assayed by stable isotope dilution capillary gas chromatography-MS as previously described (23,24).

Analysis of liver choline metabolites. Liquid chromatography-MS was used to measure free choline, betaine, and dimethylglycine (25) as well as phosphatidylcholine, lysophosphatidylcholine, sphingomyelin, phosphocholine, and glycerophosphocholine (26) with modifications based on our instrumentation (11).

Statistical analysis. Statistical analysis was conducted using a 2-way ANOVA with interactions of interest included in the initial model (JMP, SAS Institute). Effects were considered significant at $P \leq 0.05$.

Results

***Mthfd1^{gt/+}* genotype is associated with higher liver choline, betaine, and dimethylglycine.** As shown in Table 1, the *Mthfd1^{gt/+}* genotype was associated with higher concentrations of choline, betaine, and dimethylglycine in liver tissue. The livers of *Mthfd1^{gt/+}* mice had 95% higher choline ($P = 0.005$) as well as ~50% higher dimethylglycine ($P = 0.004$) and betaine ($P = 0.013$) relative to *Mthfd1^{+/+}* mice. *Mthfd1^{gt/+}* mice also had 43% lower concentrations of liver glycerophosphocholine ($P = 0.002$) than *Mthfd1^{+/+}* mice (Table 1). Notably, the FD diet did not perturb liver choline metabolites in either *Mthfd1^{+/+}* or *Mthfd1^{gt/+}* mice, nor were any gene \times diet interactions detected ($P > 0.10$) (Table 1).

Plasma biomarkers of 1-C metabolism and transsulfuration are altered in *Mthfd1^{gt/+}* mice. Plasma concentrations of the choline metabolites, dimethylglycine ($P = 0.004$) and methylglycine ($P < 0.001$), were higher in *Mthfd1^{gt/+}* than in *Mthfd1^{+/+}* mice as were plasma concentrations of homocysteine ($P = 0.002$) and cysteine ($P < 0.001$), a product of the transsulfur-

TABLE 1 Hepatic choline metabolites in *Mthfd1^{+/+}* and *Mthfd1^{gt/+}* mice after 5 wk of consuming the control or FD diet¹

Metabolite	<i>Mthfd1^{+/+}</i>			<i>Mthfd1^{gt/+}</i>			<i>P</i> value of effect	
	Control	FD	All	Control	FD	All	Diet	Genotype
<i>n</i>	10	10	20	10	10	20		
Choline, nmol/g	346 ± 273	248 ± 189	299 ± 236	621 ± 195	552 ± 289	585 ± 245	ns	0.005
Betaine, nmol/g	418 ± 202	331 ± 180	376 ± 192	618 ± 138	563 ± 262	589 ± 209	ns	0.013
Dimethylglycine, nmol/g	38 ± 13	38 ± 12	38 ± 13	54 ± 14	54 ± 15	54 ± 12	ns	0.004
Glycerophosphocholine, nmol/g	173 ± 77	225 ± 137	198 ± 110	99 ± 22	125 ± 27	113 ± 28	ns	0.002
Phosphocholine, nmol/g	409 ± 215	425 ± 287	417 ± 245	409 ± 176	268 ± 87	335 ± 151	ns	ns
Phosphatidylcholine, μmol/g	17.2 ± 2.75	17.1 ± 2.22	17.1 ± 2.44	17.2 ± 1.87	17.5 ± 0.97	17.4 ± 1.43	ns	ns
Sphingomyelin, nmol/g	567 ± 178	626 ± 151	595 ± 164	738 ± 125	657 ± 175	695 ± 155	ns	0.05
Lysophosphatidylcholine, nmol/g	506 ± 152	498 ± 142	502 ± 144	523 ± 118	570 ± 112	548 ± 114	ns	ns

¹ Data are mean ± SD. Data were analyzed using a 2-way ANOVA. *P* ≤ 0.05 was considered significant; ns, not significant, *P* > 0.10. No significant genotype × diet interactions were detected, *P* > 0.10. FD, folate deficient.

ation pathway (Table 2). The higher plasma homocysteine was more pronounced in *Mthfd1^{gt/+}* mice fed the FD diet (*P*-interaction = 0.08) (Table 2). Conversely, lower concentrations of plasma methionine (40% lower; *P* < 0.001), serine (17% lower; *P* = 0.003), and cystathionine (17% lower; *P* = 0.01) were detected in *Mthfd1^{gt/+}* mice than in the *Mthfd1^{+/+}* mice (Table 2).

Diet modestly affected the concentrations of circulating biomarkers in both *Mthfd1^{gt/+}* and *Mthfd1^{+/+}* mice; mice fed the FD diet had higher plasma concentrations of homocysteine (*P* < 0.001) and cystathionine (*P* < 0.001) relative to those fed the control diet (Table 2).

Discussion

This study demonstrates that reduced expression of the *Mthfd1* gene has significant metabolic consequences for folate, choline, methionine, and transsulfuration biochemistry (Fig. 1) and lends further support to the proposed use of *Mthfd1^{gt/+}* mice as a model for human *MTHFD1* insufficiency. Notably, the observed metabolic effects of the *Mthfd1^{gt/+}* genotype on 1-C plasma metabolites are more striking in the present study than those observed in our initial study (12); conversely, diet had a smaller effect. These disparities may have arisen from differences in mouse feeding and/or diet content. In the previous study (12), mice were feed deprived for 24 h prior to tissue harvest compared with 12 h in the current study. The longer feed deprivation period employed in the previous study (12) may have attenuated

some of the metabolic consequences of *Mthfd1* deficiency, thereby explaining the greater effect of the *Mthfd1^{gt/+}* genotype in the present study. Similarly, in our previous study (12), the diet lacked both folate and choline as opposed to folate only in the current study. The greater restriction of methyl-nutrients in our previous study (12) may have exacerbated some of the metabolic consequences of the dietary treatment, thereby explaining the smaller effect of diet in the present study.

The *Mthfd1^{gt/+}* mouse has been shown to exhibit a functional impairment in 1-C metabolism as liver *S*-adenosylmethionine (AdoMet) concentrations are reduced, presumably due to reduced AdoMet synthesis through the methionine cycle (12). Here, we observed altered methionine metabolism in the *Mthfd1^{gt/+}* mouse in the form of elevated circulating homocysteine and decreased circulating methionine relative to the *Mthfd1^{+/+}* mouse. These findings collectively indicate that disruptions in C1THF synthase activity reduce the production of 5,10-methyleneTHF and ultimately 5-methylTHF, the folate coenzyme that participates in the remethylation of homocysteine to methionine (Fig. 1) (2,19,27).

The serine hydroxymethyltransferase reaction provides an alternative route to 5,10-methyleneTHF synthesis via C1-THF synthase. Serine hydroxymethyltransferase transfers the C3 of serine to THF generating 5,10-methyleneTHF and glycine (28,29). In the present study, *Mthfd1^{gt/+}* mice had lower concentrations of circulating serine relative to *Mthfd1^{+/+}* mice, suggesting increased use of serine as source of 1-Cs for the methionine cycle and/or nuclear thymidylate biosynthesis (12) (Fig. 1).

TABLE 2 Metabolic profile of plasma from *Mthfd1^{+/+}* and *Mthfd1^{gt/+}* mice after 5 wk of consuming the control or FD diet¹

Metabolite	<i>Mthfd1^{+/+}</i>			<i>Mthfd1^{gt/+}</i>			<i>P</i> value of effect	
	Control	FD	All	Control	FD	All	Diet	Genotype
<i>n</i>	10	10	20	10	10	20		
Homocysteine, μmol/L	4.9 ± 0.6	8.0 ± 1.3	6.5 ± 1.9	6.1 ± 1.3	11.4 ± 3.6	8.8 ± 3.8	<0.001	0.002
Cystathionine, mmol/L	1.07 ± 0.27	1.49 ± 0.38	1.28 ± 0.38	0.89 ± 0.16	1.22 ± 0.20	1.06 ± 0.25	<0.001	0.01
Cysteine, μmol/L	228 ± 29	227 ± 32	228 ± 30	300 ± 52	285 ± 35	292 ± 48	ns	<0.001
Methionine, μmol/L	66 ± 29	63 ± 22	64 ± 25	37 ± 14	41 ± 14	39 ± 14	ns	<0.001
α-Aminobutyric acid, μmol/L	5.10 ± 2.41	7.65 ± 4.86	6.38 ± 3.96	8.29 ± 3.00	8.25 ± 4.84	8.27 ± 3.92	ns	ns
Glycine, μmol/L	215 ± 44	208 ± 30	211 ± 37	204 ± 32	214 ± 40	209 ± 36	ns	ns
Serine, μmol/L	141 ± 27	152 ± 29	147 ± 28	117 ± 24	125 ± 18	121 ± 21	ns	0.003
Dimethylglycine, μmol/L	7.6 ± 2.6	9.4 ± 4.9	8.5 ± 3.9	12.0 ± 2.5	12.0 ± 3.0	12.0 ± 2.7	ns	0.004
Methylglycine, μmol/L	1.39 ± 0.58	1.91 ± 0.87	1.65 ± 0.77	2.39 ± 0.62	2.69 ± 0.76	2.54 ± 0.69	ns	<0.001

¹ Data are mean ± SD. Data were analyzed using a 2-way ANOVA. *P* ≤ 0.05 was considered significant; ns, not significant, *P* > 0.10. No significant genotype × diet interactions were detected, *P* > 0.10, with the exception that plasma homocysteine tended to be higher in *Mthfd1^{gt/+}* mice fed the FD diet compared with *Mthfd1^{+/+}* fed either diet or *Mthfd1^{gt/+}* fed the control diet. *P*-interaction = 0.08. FD, folate deficient.

Mthfd1^{gt/+} mice also had higher concentrations of liver choline, betaine, and dimethylglycine and plasma dimethylglycine and methylglycine and lower concentrations of hepatic glycerophosphocholine relative to *Mthfd1*^{+/+} mice. These alterations in choline metabolism suggest increased catabolism of glycerophosphocholine, a degradative productive of phosphatidylcholine, among *Mthfd1*^{gt/+} mice to meet the greater demand for choline-derived 1-C groups: glycerophosphocholine → choline → betaine → dimethylglycine → methylglycine. Betaine is an alternative to 5-methylTHF for the remethylation of homocysteine to methionine and previous studies in mice (8) and humans (9–11) have demonstrated the increased use of choline as a methyl donor under conditions of folate insufficiency. Notably, the decrease in liver glycerophosphocholine induced by the *Mthfd1*^{gt/+} genotype was of nearly the same magnitude as that observed in a study in which mice were fed a folate-deficient diet for 12 mo (6), providing additional evidence of the adverse effects of impaired MTHFD1 activity on choline status. The purported increased use of choline-derived methyl groups under conditions of *Mthfd1* deficiency is also consistent with data from human studies (19,20), which collectively indicate a higher dietary requirement for choline among individuals with deleterious *MTHFD1* SNPs.

Disturbances in the methionine cycle due to the *Mthfd1*^{gt/+} genotype appear to have important implications for transsulfuration biochemistry (Fig. 1). *Mthfd1*^{gt/+} mice had decreased plasma concentrations of cystathionine, which is produced from homocysteine by cystathionine β-synthase (CBS), the regulatory enzyme in the transsulfuration pathway (30,31). Because AdoMet is required to activate CBS (31,32), diminished AdoMet, as seen in the livers of *Mthfd1*^{gt/+} mice (12), would be expected to result in concurrent decreases in the specific activity of CBS, thereby attenuating the conversion of homocysteine to cystathionine and conserving homocysteine for the production of AdoMet. As the precursor to cysteine, which is the end product of the transsulfuration pathway, diminished availability of cystathionine might predict diminished levels of cysteine (33). Nonetheless, circulating cysteine was higher in *Mthfd1*^{gt/+} mice than in *Mthfd1*^{+/+} mice. We suggest that, similar to what is observed with other nutrients (34), extrahepatic organs are acting to supply the *Mthfd1*^{gt/+} liver with cysteine, which can be further metabolized to glutathione, a major reductive agent within the body that is used to combat oxidative stress (35,36).

In this study, decreased MTHFD1 activity had a greater impact on 1-C metabolism compared with the FD diet and there was no interaction between the *Mthfd1* genotype and reduced dietary folate. Our findings that the FD diet did not further exacerbate the negative effects of the *Mthfd1*^{gt/+} genotype on choline and 1-C metabolic markers indicate that the 3 enzymatic activities associated with MTHFD1 are not highly dependent on intracellular folate concentrations as has been observed for other folate-dependent enzymes (37).

The comprehensive pathway alterations to choline, folate, and methionine metabolism observed in *Mthfd1*^{gt/+} mice are notably similar to alterations associated with the *MTHFD1* G1958A polymorphism and lend further support to the use of *Mthfd1*^{gt/+} mice as a model of perturbed folate- and choline-dependent 1-C metabolism and of heritable human *MTHFD1* deficiencies. Overall, the results of this study provide important insights into the metabolic changes that would be expected to arise from human *MTHFD1* insufficiency, such as in the G1958A and other recently identified *MTHFD1* SNPs (21). The study results may also inform dietary treatment approaches such as the

need for a higher choline intake among individuals with deleterious *MTHFD1* SNPs.

Acknowledgments

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